The effects of verapamil upon noradrenaline-induced contraction of the rat isolated aorta following acute and prolonged alterations in PO_2

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1 Noradrenaline (NA; ED_{90}) caused a contraction of the rat aorta which could be separated into two components, a rapid response mediated by release of intracellular Ca^{2+} and a more slowly developing contraction which relied principally upon Ca^{2+} influx.

2 Exposure to acute (30 min) hypoxia has been previously shown to reduce the NA-induced contraction (by $28.0 \pm 2.7\%$, $n = 168$) which recovered completely upon re-oxygenation (recovery response). In the present study, prolonged exposure to hypoxia (70 h) caused a more pronounced reduction ($39.7 \pm 3.0\%$, $n = 90$) of the NA-induced contraction, but, re-oxygenation then produced incomplete recovery to $77.9 \pm 3.9\%$ ($n = 90$) of the control response.

3 Prolonged exposure to 95% O_2 caused a $36.5 \pm 3.1\%$ ($n = 42$) reduction of NA-induced contractions, whereas prolonged exposure to 21% O_2 only caused a small ($12.6 \pm 3.4\%$, $n = 6$) depression of these responses.

4 The component of the NA-induced contraction mediated by release of intracellular Ca^{2+} is $39.8 \pm 1.3\%$ ($n = 83$) of the NA contraction in Ca-containing Krebs solution and was previously found to be unaffected by acute hypoxia. However, following prolonged exposure to either hypoxia or 21% O_2 , this component only reached $30.7 \pm 2.2\%$ ($n = 32$) or $28.3 \pm 0.9\%$ ($n = 6$) of the control response, respectively. Prolonged exposure to 95% O_2 caused a more pronounced reduction of this component of contraction which then reached $19.1 \pm 2.1\%$ ($n = 12$) of the control response.

5 Verapamil (10 nM–10 μ M) produced similar concentration-dependent reductions of NA-induced contractions elicited during control conditions or acute hypoxia; under these conditions, 1 μ M verapamil caused a $34.1 \pm 6.9\%$ ($n = 6$) and a $41.8 \pm 2.9\%$ ($n = 18$) reduction of these responses respectively. However, recovery responses caused by re-oxygenation of tissues exposed to acute hypoxia were more sensitive to verapamil which, at a concentration of 1 μ M, caused a $59.2 \pm 2.7\%$ ($n = 18$) reduction of these responses. Verapamil (10 nM–10 μ M) also caused similar pronounced concentration-dependent reductions of contractions elicited during prolonged exposure to normoxia or hyperoxia and of recovery responses obtained following re-oxygenation of tissues exposed to prolonged hypoxia; 1 μ M verapamil caused a $62.5 \pm 1.1\%$ ($n = 6$), $77.2 \pm 3.8\%$ ($n = 12$) and a $68.0 \pm 4.3\%$ ($n = 12$) reduction of these responses respectively. In contrast, contractions elicited during prolonged hypoxia were less sensitive to verapamil which at a concentration of 1 μ M only caused a $16.2 \pm 2.2\%$ ($n = 12$) reduction of these responses.

6 The present study indicates that prolonged exposure of the rat aorta to either hypoxic or oxygenated conditions causes attenuation of NA-induced contraction. However, these effects are also accompanied by changes in tissue Ca^{2+} handling which differ under each condition and might account for the observed modifications in tissue sensitivity to the calcium-entry blocker verapamil.

Introduction

Contraction of vascular muscle *in vitro* has been shown to be modulated by changes in the PO_2 of the environment surrounding the vessel. A majority of studies have investigated major systemic arteries,

chiefly the aorta, where acute exposure (<120 min) of vascular muscle to hypoxia caused a reduction of contractions to both adrenoceptor agonists and KCl, while a return to control PO_2 restored contractile

responses (Griesmer & Coret, 1960; Coret & Hughes, 1964; Detar & Bohr, 1968; Ebeigbe *et al.*, 1980a; Marriott, 1987). In contrast, Detar & Bohr (1972) found that when rabbit aorta had been exposed to hypoxia for 80 h, the magnitude of contraction produced by adrenaline was then as great as that produced under the original control conditions and that re-oxygenation produced relaxation rather than further contraction; this change in behaviour during prolonged exposure to hypoxia was termed 'adaptation'.

When rabbit aorta was exposed to acute episodes of hypoxia followed by re-oxygenation, Ebeigbe and co-workers (1980b) demonstrated a correlation between hypoxic depression of contractions to noradrenaline or KCl and a reduction in ^{45}Ca uptake. Moreover, these Po_2 -dependent modifications in contractility of the rabbit aorta could also be mimicked by a reduction in the concentration of extracellular Ca^{2+} (Ebeigbe *et al.*, 1980a). In accord with these results, a previous study (Marriott, 1987), indicated that an acute reduction in Po_2 preferentially reduced the component of the contraction of the rat aorta which was mediated by influx of extracellular Ca^{2+} and that the vasodilator actions of the Ca^{2+} entry blocker, diltiazem were modified under these conditions.

In view of the findings described above, the present study was designed to investigate whether there are changes in the utilization of activator Ca^{2+} by vascular muscle during prolonged exposure to hypoxia and whether modifications in Ca^{2+} -entry blocker activity occur under such conditions.

Methods

Cylindrical segments of thoracic aortae (8 mm in length) were obtained from male Wistar rats (200–280 g) killed by cervical dislocation. In all experiments the preparations were initially mounted on parallel stainless steel wire holders and equilibrated at 37°C for 60 min under a resting tension of 3 g in Krebs solution of the following composition (mM): NaCl 118.40, KCl 4.75, CaCl_2 2.50, MgSO_4 1.18, KH_2PO_4 1.19, NaHCO_3 25.00 and glucose 11.66, gassed with 95% O_2 plus 5% CO_2 (control conditions). EDTA (10 μM) and ascorbic acid (50 μM) were included in the Krebs solution in order to reduce the degradation of NA (Maxwell *et al.*, 1983).

In all cases, contractions to 1 μM NA were obtained at the beginning of the experiment under control conditions, repeated responses were obtained until successive peak contractions varied by less than 10% (control response). Pilot concentration-response experiments showed that 1 μM NA produced approximately 90% of the maximal response.

Experimental protocols

Acute exposure to hypoxia After equilibration, tissues were exposed to hypoxic conditions for 30 min (acute hypoxia) by gassing vigorously with 5% CO_2 in N_2 before stimulation with NA. Maintained responses were obtained and then tissues were rapidly re-oxygenated (by use of 95% O_2 /5% CO_2) and further tension changes recorded. The reproducibility of contractions under such conditions has been described previously (Marriott, 1987).

Prolonged exposure to different levels of Po_2 Following equilibration, preparations were carefully removed from their holders and placed in 10 ml containers (surface area 3.14 cm^2) and stored at 10°C for 70 h either in an atmosphere of 5% CO_2 in N_2 (prolonged hypoxia), 5% CO_2 in O_2 or 21% O_2 /5% CO_2 in N_2 .

After storage, vessels were replaced upon their tissue holders and re-equilibrated under tension at 37°C for 60 min in Krebs solution, gassed with either 5% CO_2 in N_2 (hypoxic protocol) or 5% CO_2 in O_2 (oxygenated protocols). Responses were then elicited to NA and repeated until successive peak contractions varied by less than 10%.

In the experiments conducted after prolonged hypoxia, responses to NA reached a stable peak tension after approximately 20 min (hypoxic response, see Figure 1) at which point the gassing mixture was rapidly switched to 5% CO_2 in O_2 and the tension changes were recorded for a further 30 min (recovery response, see Figure 1). The gas mixture was then changed back to 5% CO_2 in N_2 and NA was washed away from the tissues. Before further responses were elicited, tissues were equilibrated for 30 min under hypoxic conditions. Following prolonged exposure to oxygenated conditions,

Figure 1 Representative traces of contractions of the rat aorta induced by noradrenaline (NA, 1 μM) during control oxygenated conditions and following exposure to prolonged hypoxia (hypoxic response) followed by re-oxygenation (recovery response). Hypoxic preparations were exposed to hypoxia for 70 h before addition of NA.

responses to NA were obtained which also reached a stable peak tension after approximately 20 min. With the protocols described, contractions to NA following prolonged exposure to either oxygenated conditions or hypoxia with an associated recovery response upon re-oxygenation could be reproduced over a period of 8 h with less than 10% variation in size.

Investigation of the effects of Ca^{2+} -free conditions Preparations were exposed to Ca^{2+} -free conditions under control conditions by pre-incubating for 4 min with a modified Krebs solution from which Ca^{2+} had been omitted and 0.5 mM EGTA added. Contractions were then elicited to 1 μ M NA (EGTA-resistant response) and Ca^{2+} -free conditions maintained during the contraction. EGTA-resistant responses could be elicited over an 8 h period with less than 10% variation in size providing tissues were equilibrated for 30 min between successive responses in Krebs containing Ca^{2+} . EGTA-resistant responses were also elicited after incubation for 4 min in Ca^{2+} -free conditions following prolonged exposure of tissues to 95% N_2 /5% CO_2 , 95% O_2 /5% CO_2 or 21% O_2 /5% CO_2 in N_2 in the presence of Ca^{2+} . In these cases, all solutions were equilibrated under conditions appropriate to the relevant protocol.

Investigation of the effects of verapamil Verapamil was included in the bathing solution of both oxygenated and hypoxic tissues. Each concentration of verapamil was incubated for 30 min before a contraction to NA was elicited.

Measurement of oxygen tensions

Partial pressures of oxygen were continuously measured from one of the organ baths by either a Clarke-type catheter electrode (diameter 1.35 mm) positioned within 1 mm of the tissue or Clarke-type cell (Rank Bros., UK). Vigorous gassing of bathing solutions with 5% CO_2 in O_2 or 5% CO_2 in N_2 for 30 min produced oxygen tensions of 394 ± 6 ($n = 36$) and 76 ± 5 ($n = 24$) mmHg respectively. These PO_2 values represent an equilibrium, reached in approximately 5 min, between the composition of the gas mixture used to bubble the bathing medium and atmospheric air. Other studies frequently described higher and lower PO_2 values for solutions bubbled with 5% CO_2 in O_2 and N_2 respectively than those obtained in the present study. These differences probably reflect variations in gassing rate (see Ebeigbe *et al.*, 1980a). Prolonged exposure of the tissue bathing medium to atmospheres of 5% CO_2 in N_2 , 5% CO_2 in O_2 or 21% O_2 /5% CO_2 in N_2 for

70 h produced oxygen tensions measured at the end of the storage period of 40 ± 5 ($n = 27$), 387 ± 6 ($n = 32$) and 109 ± 4 mmHg ($n = 12$) respectively.

Statistical analyses

Values are presented as arithmetic means with the associated s.e.mean for the number (n) of animals examined. Statistical evaluation of results was carried out by Student's paired or unpaired t test where appropriate. Values of $P < 0.05$ were considered significant.

Drugs and chemicals

Noradrenaline bitartrate and EGTA-sodium salt were obtained from Sigma. Verapamil hydrochloride was a gift from Abbott. All other agents were obtained from BDH.

Results

The effects of acute exposure to hypoxia upon contractile responses and their sensitivity to verapamil

Under control conditions, contractions to NA of the rat aorta were found to consist of a rapid contraction followed by a slower increase in tension resulting in a maintained peak response (see Figure 1, control response). NA-induced contractions have been previously shown to be reduced during acute hypoxia ($28.0 \pm 2.7\%$, $n = 168$ reduction): in these tissues re-oxygenation caused a recovery of tone (recovery response) which reached $102.7 \pm 3.6\%$ ($n = 168$) of the control response to NA (see Marriott, 1987). In a Ca^{2+} -free Krebs solution containing 0.5 mM EGTA, NA caused a transient response (EGTA-resistant response) reaching $39.8 \pm 1.3\%$ ($n = 83$) of the control contraction to NA. However, the EGTA-resistant response has been previously shown to be unaffected by acute exposure to hypoxia ($0.9 \pm 3.2\%$ reduction ($n = 12$), Marriott, 1987).

Verapamil (10 nM–10 μ M) produced concentration-dependent reductions of NA-induced contractions elicited under control conditions, during acute hypoxia and of recovery responses obtained after re-oxygenation of tissues exposed to acute hypoxia (see Figure 2a). However, there was no significant difference between the effects of verapamil upon NA-induced responses elicited during hypoxia or control conditions, but verapamil (> 10 nM) produced significantly ($P < 0.001$) greater reductions of recovery responses (Figure 2a). For example, 1 μ M verapamil reduced the NA-induced contraction by $34.1 \pm 6.9\%$ ($n = 6$) and $41.8 \pm 2.9\%$ ($n = 18$) during control and

Figure 2 The effects of verapamil (10 nM–10 μ M) on contractions of the rat aorta induced by 1 μ M nor-adrenaline (NA) under conditions of (a) acute hypoxia (●—●) ($n = 18$), subsequent recovery upon re-oxygenation (○---○) ($n = 18$) and control conditions (○---○) ($n = 6$). (b) Under conditions of prolonged hypoxia (●—●) ($n = 12$), subsequent recovery upon re-oxygenation (○---○) ($n = 12$) and prolonged exposure to 95% O₂/5% CO₂ (○---○) ($n = 12$), or 21% O₂ (○---○) ($n = 6$). Asterisks indicate significant differences, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ from, in (a), the results obtained in hypoxic tissues and, in (b), the effects upon recovery responses. Vertical bars represent the s.e.mean.

hypoxic conditions respectively but caused a $59.2 \pm 2.7\%$ ($n = 18$) reduction of the recovery response (Figure 2a).

The effects of prolonged exposure to hypoxic, hyperoxic or normoxic conditions upon contractile responses

Preliminary experiments ($n = 6$) demonstrated that removal and replacement of tissues upon their holders caused less than 10% variation in the magnitude of NA-induced contractions or in their sensitivity to verapamil when compared to controls which were undisturbed.

In the presence of Ca²⁺ Contractions to NA following prolonged exposure to hypoxia retained a biphasic appearance similar to control responses, but were significantly reduced ($P < 0.001$), reaching at peak only $60.3 \pm 3.0\%$ ($n = 90$) of control responses obtained at the beginning of the experiment (see Figure 1, hypoxic response). This reduction was significantly ($P < 0.01$) greater than that seen after exposure to acute hypoxia. In approximately 50% of tissues, small spontaneous contractions were superimposed upon the maintained tension induced by NA during hypoxia (Figure 1a shows an example). In such cases, the magnitude of the hypoxic response was estimated as the mid-point between the maximum and minimum tensions. Re-oxygenation of tissues stimulated with NA during prolonged

hypoxia caused further contractions (recovery responses) though to tensions significantly ($P < 0.01$) lower than the control responses, reaching at peak $77.9 \pm 3.9\%$ ($n = 90$) of the control response to NA (see Figure 1, recovery response).

Following prolonged exposure to 95% O₂/5% CO₂, the shape of the responses to NA remained unchanged, but peak tensions were significantly ($P < 0.001$) smaller than control responses ($63.5 \pm 3.1\%$ ($n = 42$) of the control response). This reduction in the NA-induced contraction was not significantly different from that seen following exposure of tissues to prolonged hypoxia.

Contractions to NA following prolonged exposure to 21% O₂/5% CO₂ in N₂ reached $87.4 \pm 3.4\%$ ($n = 6$) of the control response: the reduction incurred was significantly ($P < 0.05$ and 0.01 respectively) smaller than those observed following prolonged exposure to hypoxia or 95% O₂/5% CO₂ (see above).

Under Ca²⁺-free conditions Exposure to prolonged hypoxia caused a reduction of the EGTA-resistant response, to $30.7 \pm 2.2\%$ ($n = 32$) of the control response to NA obtained in the presence of Ca²⁺. However, re-oxygenation did not produce any change in the EGTA-resistant response elicited during hypoxia. The reduction of the EGTA-resistant response following prolonged exposure to 21% O₂/5% CO₂ in N₂ to $28.3 \pm 0.9\%$ ($n = 6$) of the control response to NA was not significantly different from that seen following prolonged hypoxia. However, EGTA-resistant responses elicited in tissues exposed to either prolonged hypoxia or 21% O₂/5% CO₂ were significantly ($P < 0.001$ and $P < 0.05$ respectively) smaller than those elicited under control conditions. Prolonged exposure to 95% O₂/5% CO₂ caused a significantly greater depression of the EGTA-resistant response ($P < 0.01$ from hypoxic and 21% O₂ results) to $19.1 \pm 2.1\%$ ($n = 12$) of the control response to NA obtained in the presence of Ca²⁺.

In the presence of verapamil Verapamil (10 nM–10 μ M) also caused a concentration-dependent reduction of contractions to NA in tissues exposed to either prolonged hypoxia or prolonged oxygenated conditions (see Figure 2b). However, there was no consistent significant difference between the effects of verapamil upon NA-induced contractions elicited following prolonged exposure to 95% O₂/5% CO₂ or 21% O₂/5% CO₂ in N₂ or recovery responses obtained following re-oxygenation of tissues previously exposed to prolonged hypoxia (see Figure 2b); 1 μ M verapamil caused a $77.2 \pm 3.8\%$ ($n = 12$), $62.5 \pm 1.1\%$ ($n = 6$) and a $68.0 \pm 4.3\%$

Figure 3 Representative traces of contractions of the rat aorta to noradrenaline (NA, $1 \mu\text{M}$) during hypoxia (70 h) and following re-oxygenation (recovery). Contractions elicited in the presence of verapamil (Ver, 10 nM– $10 \mu\text{M}$) are also shown. Time and tension scales apply to all contractions.

($n = 12$) reduction of these responses respectively. In contrast, NA-induced contractions elicited during prolonged hypoxia were significantly ($P < 0.001$) less sensitive to verapamil than those evoked under the oxygenated conditions described above (see Figure 2b). For example, $1 \mu\text{M}$ verapamil only reduced the NA-induced contraction during prolonged hypoxia by $16.2 \pm 2.2\%$ ($n = 12$). Examples of the differences between the effects of verapamil (10 nM– $10 \mu\text{M}$) upon contractions induced by NA during prolonged hypoxia with subsequent re-oxygenation are shown in Figure 3.

Comparisons between the effects of verapamil in tissues exposed to acute and prolonged changes in P_{O_2}

NA-induced contractions elicited in tissues subjected to prolonged 21% O_2 /5% CO_2 in N_2 (prolonged normoxia) were significantly ($P < 0.01$) more sensitive to verapamil ($> 100 \text{ nM}$) than those elicited under control conditions (compare b and a in Figure 2). For example, $1 \mu\text{M}$ verapamil caused a $62.5 \pm 1.1\%$ ($n = 6$) reduction of NA-induced contractions following prolonged normoxia (see Figure 2b) yet only caused a $34.1 \pm 6.9\%$ ($n = 6$) reduction of these contractions under control conditions (Figure 2a). In contrast, during prolonged hypoxia NA-induced contractions were significantly less sensitive to verapamil ($P < 0.01$, $< 100 \text{ nM}$; $P < 0.001$, $> 100 \text{ nM}$) than under corresponding conditions of acute hypoxia (compare b and a in Figure 2); $1 \mu\text{M}$ verapamil reduced these responses by $41.8 \pm 2.9\%$

($n = 18$, Figure 2a) and $16.2 \pm 2.2\%$ ($n = 12$, Figure 2b) respectively. However, recovery responses obtained following re-oxygenation of tissues exposed to either acute or prolonged hypoxia showed similar sensitivities to verapamil (compare a and b in Figure 2); under these conditions $1 \mu\text{M}$ verapamil caused a $59.2 \pm 2.7\%$ ($n = 18$, Figure 2a) and a $68.0 \pm 4.3\%$ ($n = 12$, Figure 2b) reduction respectively.

Qualitatively similar results to those described in the present study have also been obtained using the Ca^{2+} -entry blockers diltiazem, nifedipine and flunarizine which are from chemical sub-groups distinct from verapamil.

Discussion

The present study has demonstrated that prolonged alterations in oxygen tension can modify the magnitude of NA-induced contractions in the rat isolated aorta. These findings extend a previous study in which acute hypoxia was found to attenuate contractility in this vessel (Marriott, 1987). Since NA-induced contractions of the rat aorta can be resolved into components which are mediated by either release of intracellular Ca^{2+} or influx of Ca^{2+} from the extracellular environment (Yamashita *et al.*, 1977), the P_{O_2} -dependency of these processes has also been examined in the present study by use of verapamil or Ca^{2+} -deprivation, procedures which reduce the availability of extracellular Ca^{2+} for contraction.

Mechanisms responsible for P_{O_2} -dependent changes in contractility

Several mechanisms have been proposed in order to account for hypoxic depression of vascular contractility and these include release of endogenous vasodilators, activation of oxygen receptors, a reduction in available energy, and a reduction in membrane permeability to ions important for contraction (Pittman, 1981).

In the present investigation and a previous study (Marriott, 1987), the reduction in NA-induced contractions of the rat aorta observed during acute hypoxia appeared to be consistent with a preferential attenuation of contractile processes mediated by influx of extracellular Ca^{2+} . Contraction thought to rely exclusively upon release of intracellular Ca^{2+} being unaffected by these hypoxic conditions (see Marriott, 1987). In addition, the complete recovery of NA-induced contractions resulting from re-oxygenation of tissues exposed to acute hypoxia, seen in this and a previous study (Marriott, 1987), appear to result from activation of processes which depend upon Ca^{2+} influx, since recovery responses were abolished in the absence of extracellular Ca^{2+} .

These observations support the findings of Ebeigbe (1982) who described a reduction and an increase in ^{45}Ca uptake of NA-stimulated rabbit aorta during acute hypoxia and following re-oxygenation respectively.

The possibility that changes in Ca^{2+} availability might mediate modifications in vascular contractility following prolonged alterations in oxygen tension has also been examined in the present study. However, in order to estimate the degenerative effects of tissue storage upon contractility, preparations were also maintained for a prolonged period at a PO_2 normally encountered in arterial blood (normoxia produced by storage in an atmosphere of 21% O_2 /5% CO_2 in N_2). Such normoxic conditions were found to reduce NA-induced contractions mediated by both intra- and extracellular Ca^{2+} and these reductions are taken to reflect the effects of storage procedures upon tissue viability.

Prolonged exposure of tissues to hypoxic or normoxic conditions produced similar reductions in contractions mediated by intracellular Ca^{2+} , indicating that such effects result from degenerative processes during storage.

However, in the presence of extracellular Ca^{2+} , prolonged exposure to hypoxia caused a reduction in contractility, in excess of that expected to result from storage procedures. Consequently, it is possible that exposure of rat aorta to prolonged periods of hypoxia produces a preferential interference with contractile processes which depend upon Ca^{2+} influx. It is not clear if these processes are similar to those responsible for the hypoxia-induced reduction in ^{45}Ca uptake of NA-stimulated rabbit aorta found by Ebeigbe and co-workers (1980b).

The effects of prolonged exposure to hypoxia upon contractility also appeared to be readily reversible in the present study, since re-oxygenation of tissues previously exposed to prolonged hypoxia caused a recovery of NA-induced contraction. As these recovery contractions were not apparent in a Ca^{2+} -free medium, such responses were also likely to depend upon influx of extracellular Ca^{2+} . In addition, these recovery contractions were of a similar size to responses elicited in tissues exposed to prolonged normoxia. This indicates that degenerative processes operating during storage prevented complete recovery of responses to control values as observed in corresponding tissues exposed to acute hypoxia (see Marriott, 1987 and above).

It is interesting that the findings from the present study contrast markedly with those of Detar & Bohr (1968, 1972), who obtained a paradoxical relaxation to oxygen in adrenaline-stimulated rabbit aorta exposed to prolonged hypoxia. The reasons for these discrepancies remain obscure. However, differences in study design, namely species, form of preparation

and agonist employed might be critical in producing the hypoxic 'adaptation' in vascular contractility described by Detar & Bohr (1972).

The present study also examined the effects of prolonged exposure to a high oxygen tension (hyperoxygenation) upon NA-induced contraction of the rat aorta. In contrast to the preferential depression of contractions mediated by extracellular Ca^{2+} observed during prolonged hypoxia, prolonged hyperoxygenation caused similar reductions of contractions mediated by either intra- or extracellular Ca^{2+} ; these reductions were greater than the expected loss of tissue viability due to storage alone. It is therefore possible that prolonged exposure of rat aorta to a high oxygen tension produces a general reduction of contractile processes, by a mechanism differing from that operating during prolonged hypoxia. It is, however, impossible to speculate upon the nature of such processes at the present time.

Mechanisms of PO_2 -dependent modifications in verapamil sensitivity

Verapamil is known to block stimulated entry of Ca^{2+} into vascular muscle, whereas, passive leaks of Ca^{2+} into these cells appear to be insensitive to this agent (Flaim, 1982). Vascular contractility has also been shown to be reduced by verapamil through mechanisms independent of calcium entry blockade (Raddino *et al.*, 1987), but these actions are associated with the use of higher concentrations ($> 10 \mu\text{M}$) than those utilised in the present study (Triggle, 1982). However, in the rat aorta the effects of selective blockade of Ca^{2+} entry processes by verapamil need not be identical to those of extracellular Ca^{2+} withdrawal, since different pathways for activated Ca^{2+} entry do not appear to be equally sensitive to verapamil (Marriott, 1988).

The present study has demonstrated that the ability of verapamil to block NA-induced contractions of the rat aorta is modified by both acute and prolonged changes in PO_2 . A change in vascular sensitivity to calcium entry blockade might result from (a) differences in the relative contribution of Ca^{2+} from an intracellular source to contraction, and/or (b) changes in the populations of channels gating Ca^{2+} entry (Van Breemen *et al.*, 1982), or (c) through modification in the binding of the calcium entry blocker to its active site. The latter mechanism would appear unlikely in the present study, since calcium entry blockers from different chemical subgroups appear to bind to unique sites (Spedding, 1985), yet all appear to undergo similar changes in activity following alterations in PO_2 (Marriott, 1987).

Clearly, those vascular contractions which are mediated by release of intracellular Ca^{2+} should be

insensitive to calcium entry blockade, unless release processes are triggered by influx of extracellular Ca^{2+} . Previous studies have demonstrated that EGTA-resistant responses of the rat aorta are indeed resistant to verapamil, supporting the suggestion that these responses are mediated by intracellular Ca^{2+} (Heaslip & Rahwan, 1983; Marriott, 1988). Nevertheless, it is not certain that the relative contribution of Ca^{2+} from an intracellular source ultimately determines the verapamil sensitivity of sustained NA-induced contractions elicited in the presence of Ca^{2+} , since in the rat aorta, intracellular Ca^{2+} is only transiently involved during the early part of NA-induced contractions (Yamashita *et al.*, 1977).

Although in the present study verapamil generally produced larger reductions of contractions, in which the contribution of intracellular Ca^{2+} was depressed (i.e. following storage under oxygenated conditions), no graded relationship between the sensitivity of contractions to verapamil and their dependency upon intracellular Ca^{2+} could be found. For example, the relative contribution of intracellular Ca^{2+} to NA-induced contraction appeared similar during both acute and prolonged hypoxia; EGTA-resistant responses amounted to approximately 50% of the size of corresponding contractions in the presence of Ca^{2+} in each case. However, tissues were significantly more sensitive to verapamil under the former conditions. Consequently, such findings indicate that in the rat aorta the relative contribution of

intracellular Ca^{2+} to contraction is not a major determinant of verapamil sensitivity following PO_2 changes.

It is therefore possible that a modification in systems gating Ca^{2+} entry are responsible for the PO_2 -dependent changes in verapamil sensitivity observed in the present study. However, the nature of such changes remain obscure. It is interesting that McGrath & Ugwu (1986) have previously indicated that oxygen tension might control the populations of channels gating Ca^{2+} entry, since they found that nifedipine-sensitive channels were more important during NA-induced contraction of the rat tail artery following an increase in O_2 content.

The present study has shown that NA-induced contractions of the rat aorta are reduced following prolonged exposure to hypoxia and oxygen. However, it is possible that different mechanisms are responsible in each case. In addition, these modifications in contractility are accompanied by changes in tissue sensitivity to verapamil, probably due to PO_2 -dependent effects upon Ca^{2+} influx pathways. Such findings may be relevant in predicting the therapeutic effects of calcium entry blocking agents in conditions involving chronic ischaemia and reoxygenation-induced vasospasm. In addition, these findings might be of value in attempts to preserve tissue viability during transplant or grafting procedures.

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